

Appendix P

Examples of “Weight-of-Evidence” Determinations

The following charts were developed, at the request of the EDSTAC, by one EDSTAC member and one STWG member (not an EDSTAC member). The Appendix is intended to give the reader a sense of how results could be interpreted, however, it does not include all possible examples. For instance, the document does not include an example of how one might handle positive *in vitro* results coupled with negative *in vivo* results in Tier 1, which might go into the “hold box.”

TIS RESPONSE/ “Weight-Of-Evidence” TABLE. These three tables (parts 1, 2, and 3) include known or expected responses to the assays included in the proposed EDSTP TIS battery. The list of endpoints include only those required. After the following: + is a positive; - is negative; +? is a possible positive response; and shaded responses are more certain than others. “Weight-of-evidence” determinations are made to determine the next steps for the chemical in question. The three possible outcomes are (1) Positive, (2) Negative, or (3) Other. Most chemicals truly exist, and <i>in vivo</i> dosage levels are provided, while a few are fictitious, generated for illustrative purposes. The substances are identified by brief descriptions.							
Part 1.							
TIS ASSAY	ENDPOINT	Anti-androgenic Fungicide	Estrogenic Toxic Substance	Estrogenic Pesticide	Anti-androgenic Drug	PCBMixture	Anti-A pesticide
IN VITRO HTPS OR BENCH ASSAYS							
HTPS ER/MVLN	ER AGONIST	-	+	+	-	-	-
ABOVE PLUS E2	ER ANTAGONIST	-	-	-	-	-	-
HTPS ER PLUS METABOLISM	METAB IS ER AGONIST	-	-	++	-	-	-
ABOVE PLUS E2	METAB IS ER ANTAGONIST	-	-	-	-	-	-
HTPS AR/CV-1	AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	AR ANTAGONIST	+	+	+	-	-	+
HTPS AR PLUS METABOLISM	METAB IS AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	METAB IS AR ANTAGONIST	++	-	++	-	-	-
HTPS TR	TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	TR ANTAGONIST	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	METAB IS TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	METAB IS TR ANTAGONIST	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	INHIBITION OF P4 OR T SYNTHESIS	-	-	-	-	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	+	+	-	-	-
	UTERINE HISTOLOGY	-	+	+	-	-	-
	BODY WEIGHT	-	-	-	-	-	-

	VAGINAL CORNIFICATION	-	+	-	-	-	-

3 DAY UIEROI TROPIC IN ADULT OVX'D RAT PLUS E2	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	-	-	-
	UTERINE HISTOLOGY	-	-	-	-	-	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	-	-	-

PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	GROWTH	-	-	-	-	-	-
	AGE AT VAGINAL OPENING	-	++	++	-	-	-
	WEIGHT AT VAGINAL OPENING	-	++	++	-	-	-
	SERUM T4	-	-	-	-	++	-
	SERUM TSH	-	-	-	-	+	-
	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	+	-	-	-
	OVARIAN WEIGHT	-	-	+	-	-	-
	THYROID HISTOLOGY	-	-	-	-	+	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ↑	-	-	-	LIVER ↑	LIV ↑

7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	-	-	-	-	+-?	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ↑	-	-	-	LIVER ↑	LIV ↑

7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT: PLUS T	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	+	-?	+	-	+-?	+
	VENTRAL PROSTATE WEIGHT	+	-?	+	++	+-?	+
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	+	-?	+	-	+-?	+
	GROWTH	-	-	+	-	+-?	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ↑	-	-	-	LIVER ↑	LIV ↑

"Weight-of-Evidence" Determination: Hold, Go to T2T, or Other		T2T	T2T	T2T	Repeat T1S T2T	T2T	T2T
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PART 2. Known or expected responses of well characterized toxicants in the EDSTP T1S battery. The list of endpoints and the expected responses of well characterized toxicants in the EDSTP T1S battery. All of the described examples would have sufficient positive responses to be triggered for T2T.

T1S ASSAY	ENDPOINT						
IN VITRO HTPS OR BENCH ASSAYS		Steroidogenesis Fungicide	Thyroid Hormone Analogue	Fungicide	Wood- derived Estrogen	Estrogenic Pesticide	Anti- thyroid Drug
HTPS ER/MVLN	ER AGONIST	-	-	-	+-	+	-
ABOVE PLUS E2	ER ANTAGONIST	-	-	-	-	-	-
HTPS ER PLUS METABOLISM	METAB IS ER AGONIST	-	-	-	-	-	-
ABOVE PLUS E2	METAB IS ER ANTAGONIST	-	-	-	-	-	-
HTPS AR/CV-1	AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	AR ANTAGONIST	-	-	-	-	-	-
HTPS AR PLUS METABOLISM	METAB IS AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	METAB IS AR ANTAGONIST	-	-	-	-	-	-
HTPS TR	TR AGONIST	-	+	-	-	-	-
ABOVE PLUS T3	TR ANTAGONIST	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	METAB IS TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	METAB IS TR ANTAGONIST	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	INHIBITION OF P4 OR T SYNTHESIS	++	-	-?	++?	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPIC IN ADULT OVX'D RAT	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	-	+	-
	UTERINE HISTOLOGY	-	-	-	-	+	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL SMEAR	-	-	-	-	-	-
3 DAY UTEROTROPIC IN ADULT OVX'D RAT PLUS E2	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	-	-	-
	UTERINE HISTOLOGY	-	-	-	-	-	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL SMEAR	-	-	-	-	-	-

PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	GROWTH	-	-?	-	-	-	+?
	AGE AT VAGINAL OPENING	+↑	-?	+↑	+?↑	+	+?
	WEIGHT AT VAGINAL OPENING	+↑	-?	+↑	+?↑	+	-
	SERUM T4	-	+↓?	-	-	-	++
	SERUM TSH	-	+↓?	-	-	-	++
	UTERINE WEIGHT WITH AND WITHOUT FLUID	+?↓	-?	+?↓	-	-	+?
	OVARIAN WEIGHT	+↑	-?	+↑	-	-	+?
	THYROID HISTOLOGY	-	+?↓	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ?↑	-?	LIVER ↑	LIVER ↑?	LIVER↑	THYROID ↑
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	-	-?	-	-	-	+?
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ?↑	-	+?↑	-	LIVER↑	+?
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT; PLUS T	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	-	-	-	+	+?
	VENTRAL PROSTATE WEIGHT	-	-	-	-	+	+?
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	+?	+?
	GROWTH	-	-?	-	-	-	+?
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ?↑	-	LIVER↑	-	LIVER↑	+?
"Weight -of-Evidence" Determination: Hold, Go to T2T, or Other		T2T	REPEAT T1S, T2T	T2T	MAY REPEAT T1S, T2T	T2T	T2T

PART 3. Known or expected responses of well characterized toxicants in the EDSTP TIS batttery. The list of endpoints

TIS ASSAY	ENDPOINT						
IN VITRO HTPS OR BENCH ASSAYS		Neurotoxic Pesticide	Plasticizer	Anti- androgeni c Fungicide	Anti- estrogenic Drug	Herbicide	Xeno- estrogen
HTPS ER/MVLN	ER AGONIST	++	++	-	-	-	++
ABOVE PLUS E2	ER ANTAGONIST	-	-	-	++	-	-
HTPS ER PLUS METABOLISM	METAB IS ER AGONIST	-	-	-	+	-	-
ABOVE PLUS E2	METAB IS ER ANTAGONIST	-	-	-	+++	-	-
HTPS AR/CV-1	AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	AR ANTAGONIST	-	-	-	-	-	-?
HTPS AR PLUS METABOLISM	METAB IS AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	METAB IS AR ANTAGONIST	-	-	++	-?	-	-
HTPS TR	TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	TR ANTAGONIST	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	METAB IS TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	METAB IS TR ANTAGONIST	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	INHIBITION OF P4 OR T SYNTHESIS	-	-?	-	-	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	++	-	++
	UTERINE HISTOLOGY	-	-	-	++	-	++
	BODY WEIGHT	+	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	++	-	+
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT PLUS E2	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	++	-	-
	UTERINE HISTOLOGY	-	-	-	++	-	-
	BODY WEIGHT	+	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	++	-	-

PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	GROWTH	+	-	-	-	-	-
	AGE AT VAGINAL OPENING	-	-	-	+?	+↓?	-
	WEIGHT AT VAGINAL OPENING	-	-	-	+?	+↓?	-
	SERUM T4	-	-	-	-	-	-
	SERUM TSH	-	-	-	-	-	-
	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	+	-	-
	OVARIAN WEIGHT	-	-	-	+	-	-
	THYROID HISTOLOGY	-	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
							-
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	+	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT; PLUS T	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	+?	++	-	-	-
	VENTRAL PROSTATE WEIGHT	-	+?	++	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	+?	+	-	-	-
	GROWTH	+	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
"Weight-of-Evidence" Determination: Hold, Go to T2T, or Other		REPEAT T1S, Hold ¹	T2T	T2T	T2T	REPEAT T1S, T2T	Other ²

Footnote (1) Of the described examples, the neurotoxic pesticide would go to the "hold box" or be studied further before it would go to T2T. Footnote (2) The xenoestrogen is only estrogenic in vitro and by injection, but was not estrogenic by oral treatment. The uterotrophic assay should be repeated with oral dosing to determine if the xenoestrogen was estrogenic by the oral route before it went to T2T.]